Investigation of *Phytophthora infestans* Population Structure in China

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Origin of the Project

Issues:

***P. infestans* population changes** were reported to be associated with the **increasing severity of late blight** outbreaks on potato.

Few *P. Infestans* population structure reported in China could be compared with data of others due to **utilization of differential detection techniques**.

Goals:

We aimed to investigate *P. infestans* population structure in China in 2015-2017, by examining its phenotypic and genotypic traits.

Provide fundamental information of *P. infestans* population structure to AsiaBlight organization.

Experimental Design

-Samples were collected at 15 locations in 8 provinces in China from 2015 to 2017

-At least 2 sampling sites at each location

-30-40 plants were sampled at each sampling site

Marker Selection

Phenotypic Marker

Mating Type

Metalaxyl Resistance

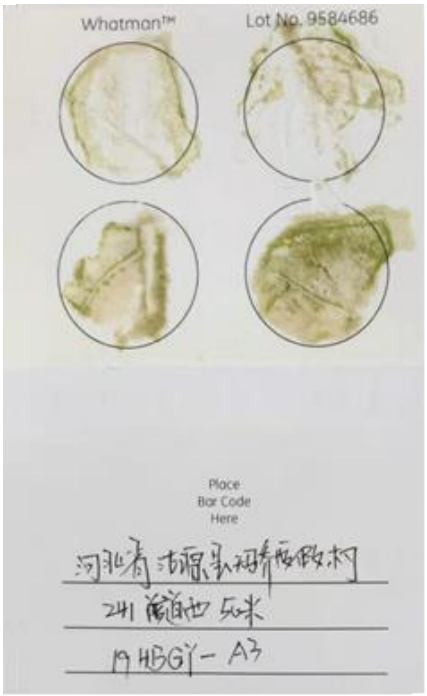
Aggressiveness Test

Genotypic Marker

Simple Sequence Repeat (12 SSR markers, developed by Li et al. 2013)

Sample Collection

Leaves with lesion area bigger than 10% of the whole leaf area were collected in plastic bags and brought back to lab for isolation



Few samples were collected with FTA cards

Isolation of *P. infestans*

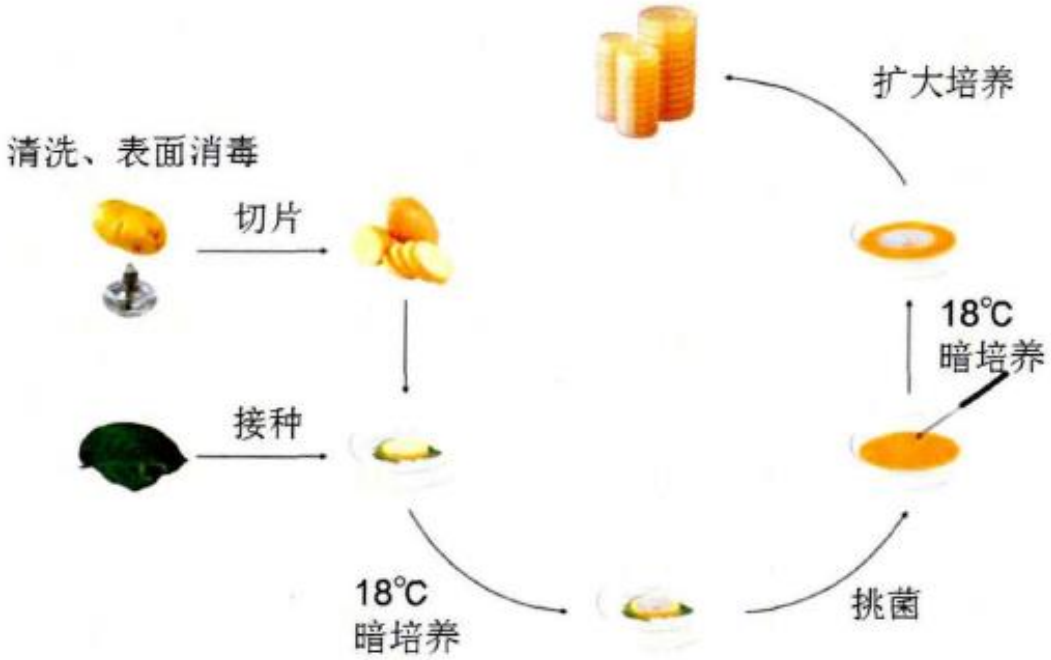


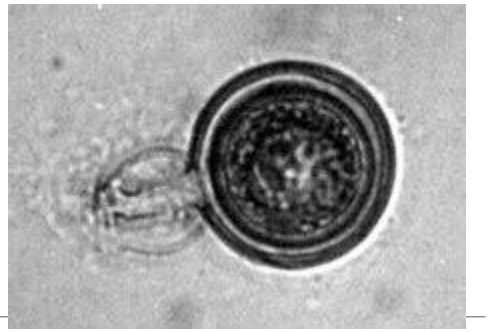
图 2.4 致病疫霉纯化方法

Fig. 2.4 *Phytophora infestans* purification method

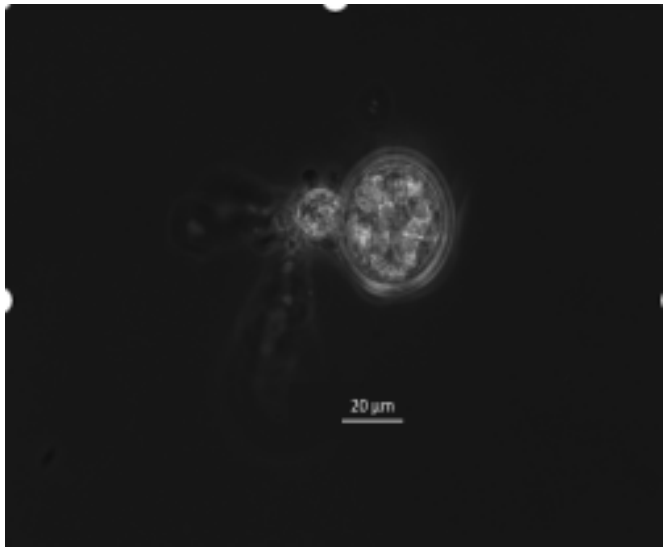
Sihui Chen. 2015. Thesis: Popupation genetic analysis of *Phytophthora infestans* in China.

Phenotyping

-Mating Type Test



Unknown and standard isolates (A1 or A2 mating type) were grown on V8 agar for 7 days



Oospore

Standard mating type was kindly provided by Dr. Weixing Shan, Northwest A&F University

Metalaxyl Resistance

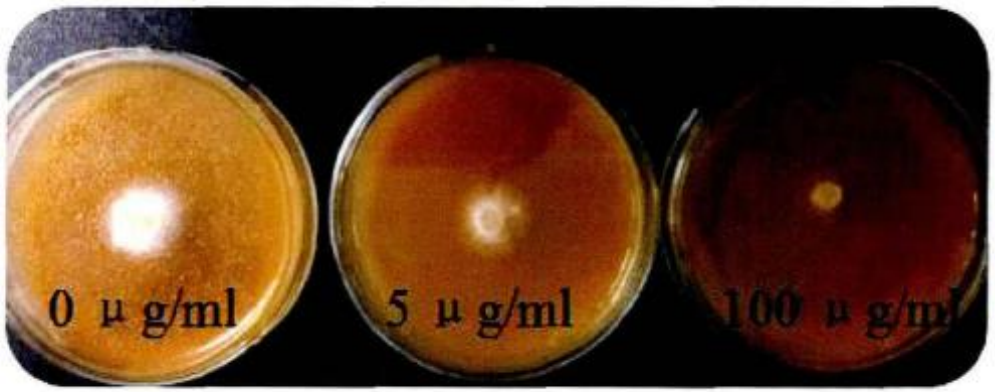


图 2.7 甲霜灵抗性检测

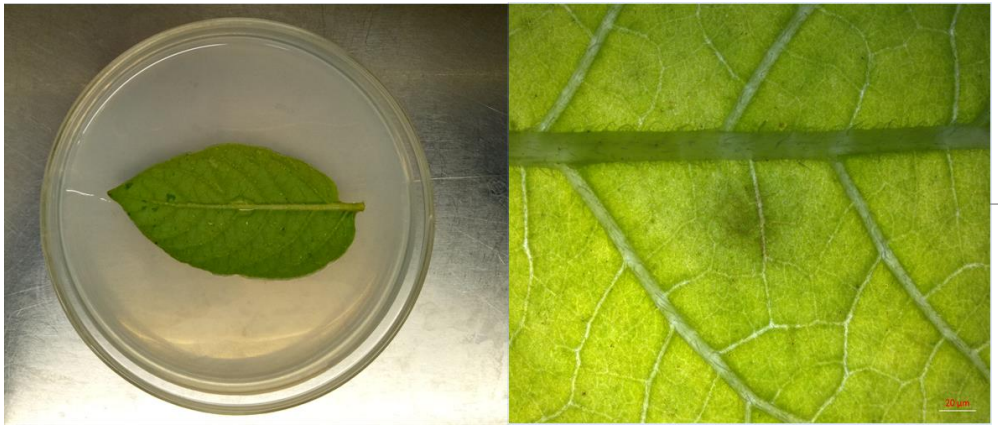
Fig. 2.7 Metalxyl sensitivity test

Resistant $M_5 > 40\% M_0$ 且 $M_{100} > 40\% M_0$;

Intermediate $M_{100} < 40\% M_0 < M_5$;

Sensitive $M_5 < 40\% M_0$ 且 $M_{100} < 40\% M_0$。

Sihui Chen. 2015. Thesis: Popupation genetic analysis of *Phytophthora infestans* in China.
Forbes G. 1997.Cips Training Manual.



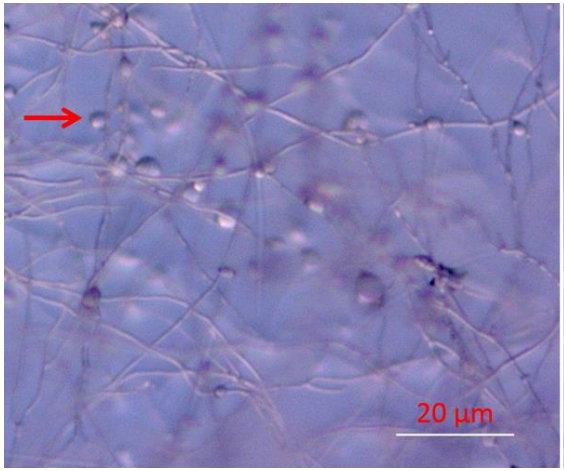
Inoculation of 20 µL（1*104/µL）
P. infestans on leaflets

Aggressiveness Test

Latency Period

Lesion Growth Rate

Number of sporangia per lesion



Contrast

Experimental observation

Aggressiveness Test

Lesion Growth Rate （mm^2/day) + Number of Sporangia per Lesion

0 day

4 day
Abaxial surface

4 day
Adaxial surface

5 day
Abaxial surface

5 day
Adaxial surface

6 day

7 day

8 day

9 day

10 day

Genotyping

DNA extraction

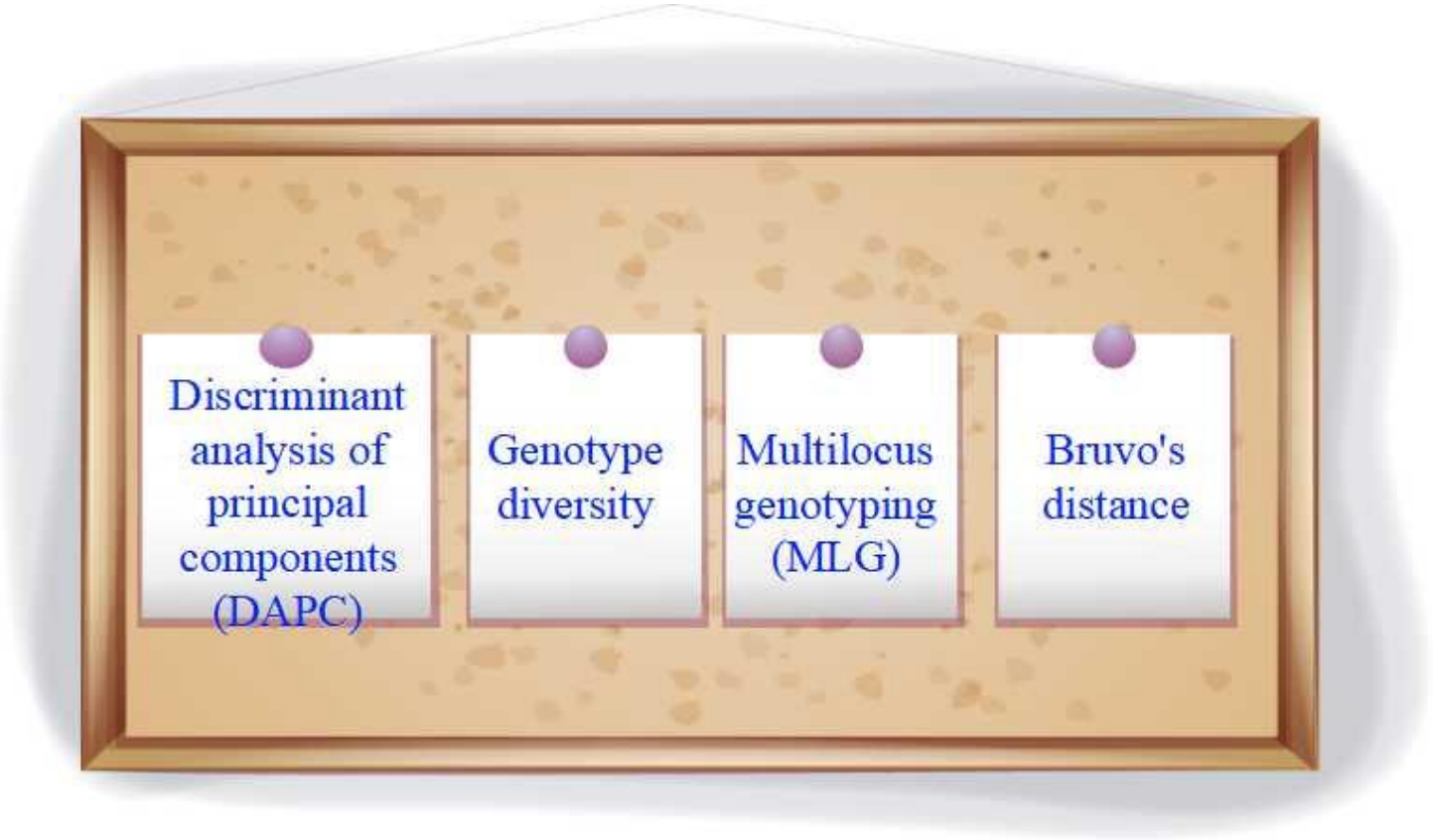
PCR reaction (12 SSR Loci primers developed by Li et al. 2013)

Electrophoresis and visualization of alleles were performed on an ABI3130 genetic analyzer (Finished by company)

Allele sizing was determined using GENEMAKER software

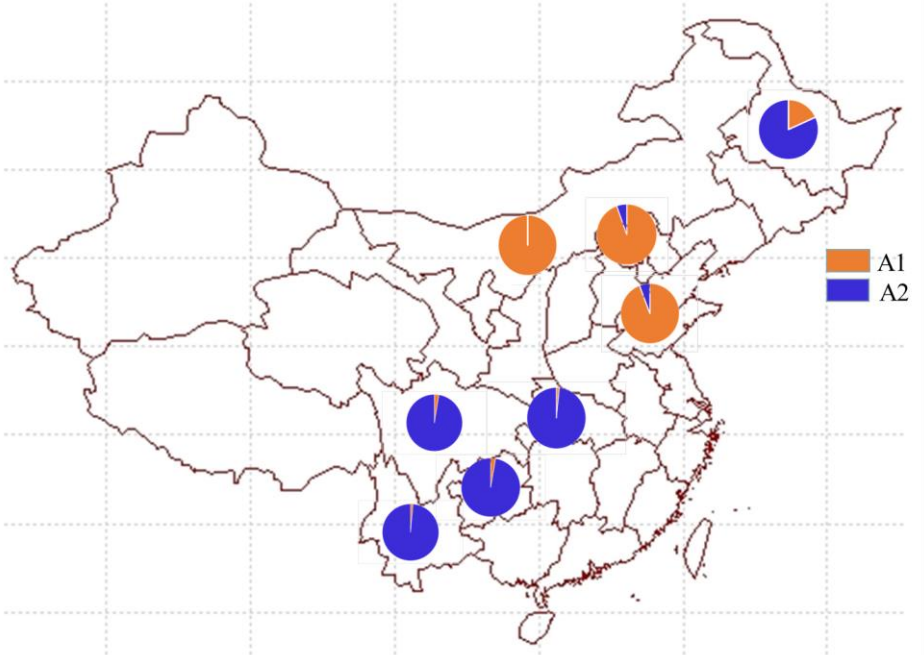
Data Analysis

R program's Poppr Package population analysis

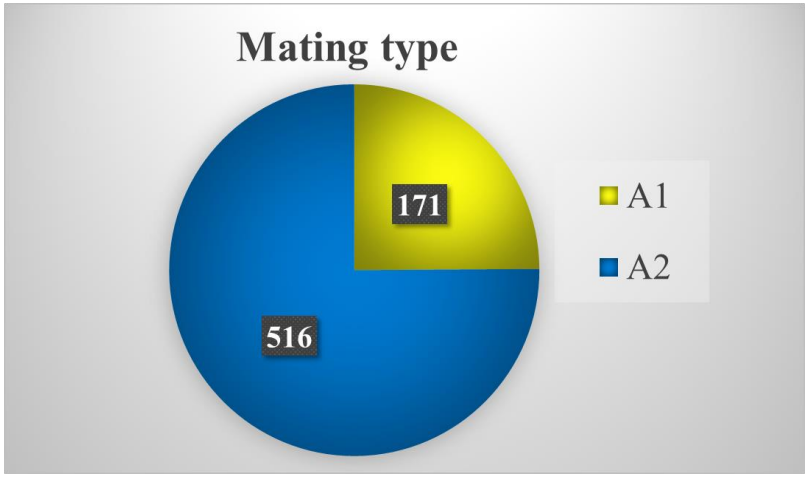


Results

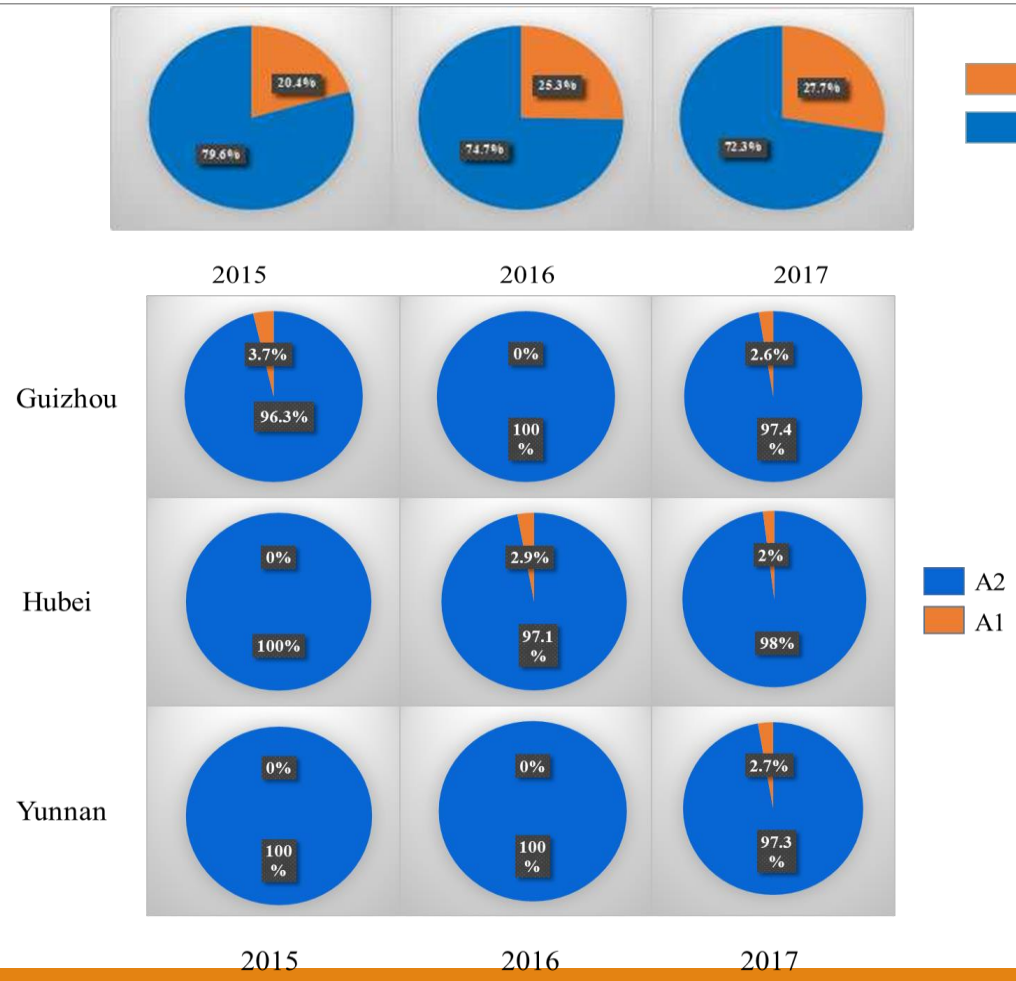
Mating type **A1** is dominant in **northern** regions
Mating type **A2** is dominant in **southern** regions



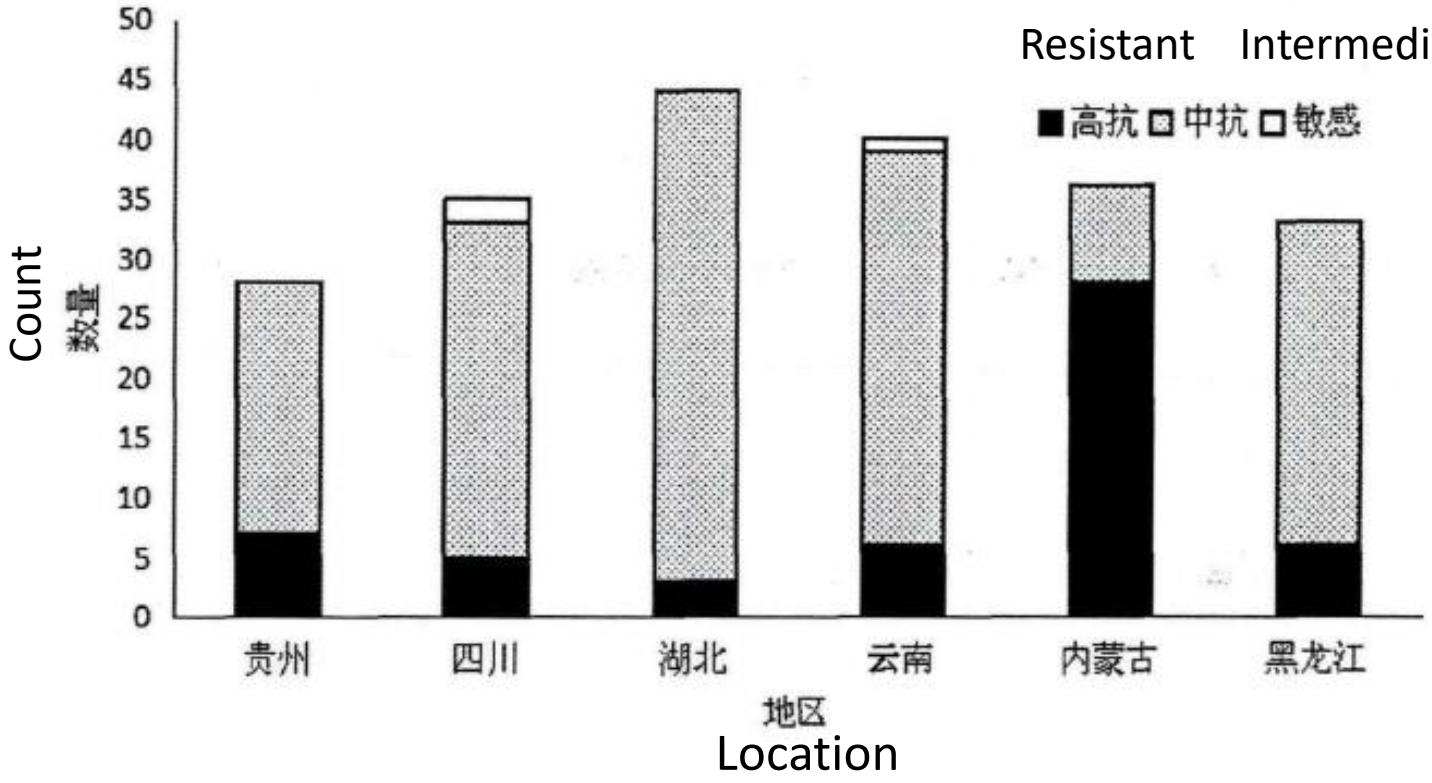
Map of China



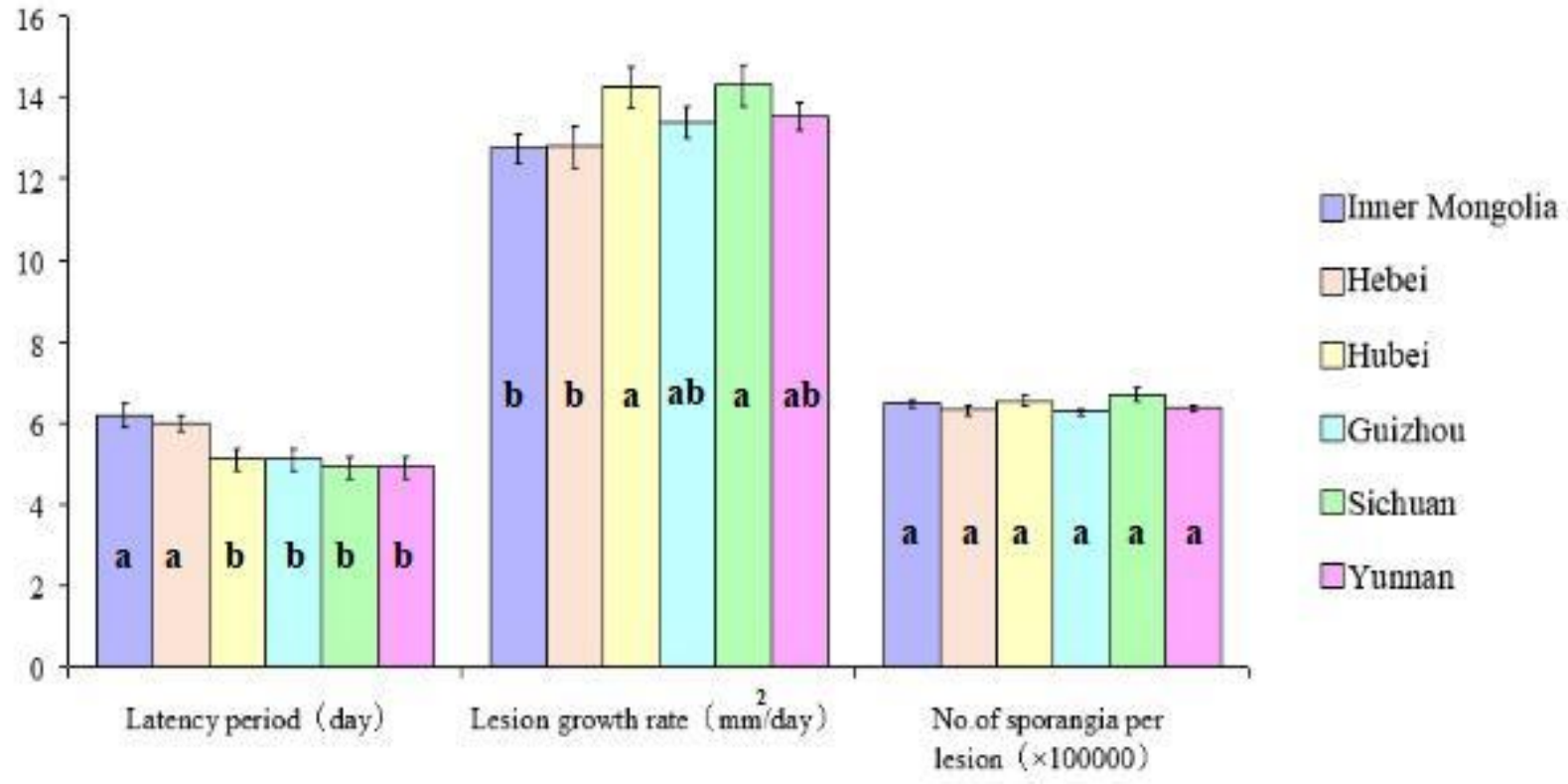
Overall, proportion of A1 mating type is increased from 2015 to 2017 A1:A2 ratio fluctuated in different provinces over time



Majority isolates in Inner Mongolia are highly resistant to Metalaxyl
Majority isolates in southern China are intermediate to Metalaxyl



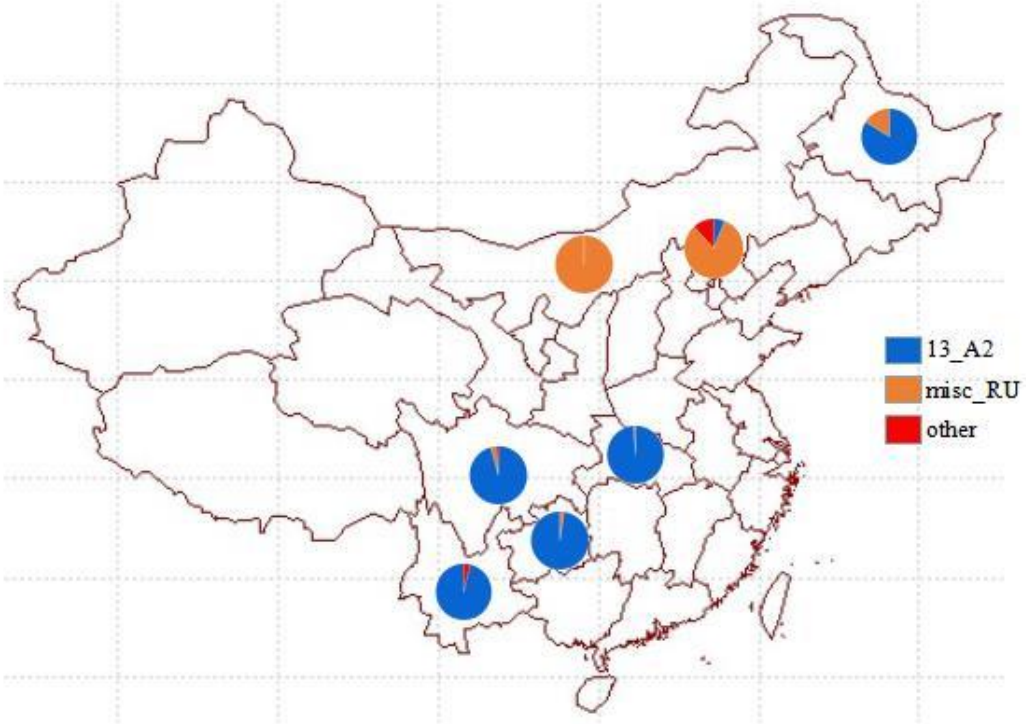
Isolates in northern regions have **longer latency period** and **slower lesion growth rate** compared with southern regions



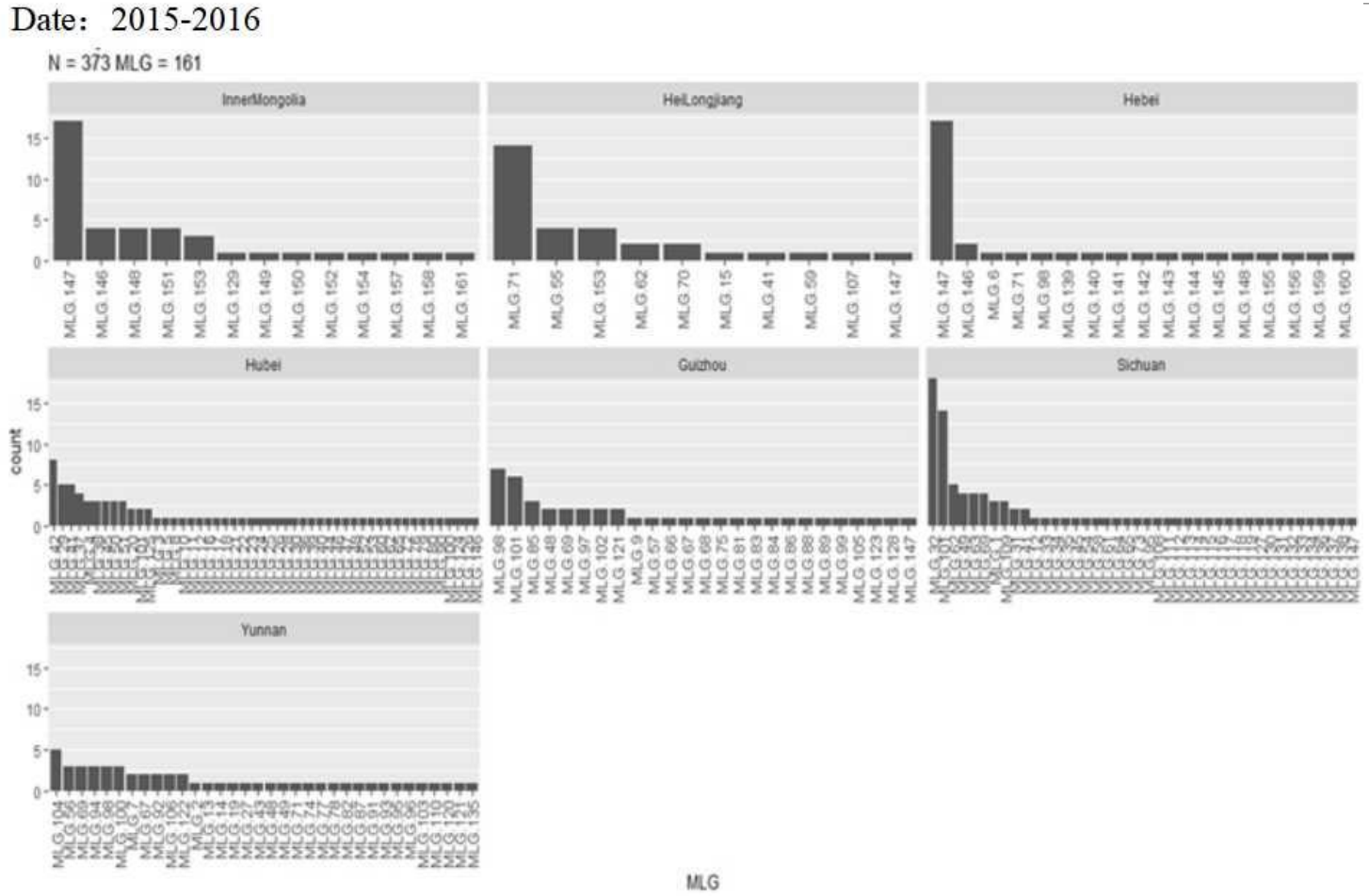
13_A2 is dominant in southern regions

mis_RU is dominant in northern regions

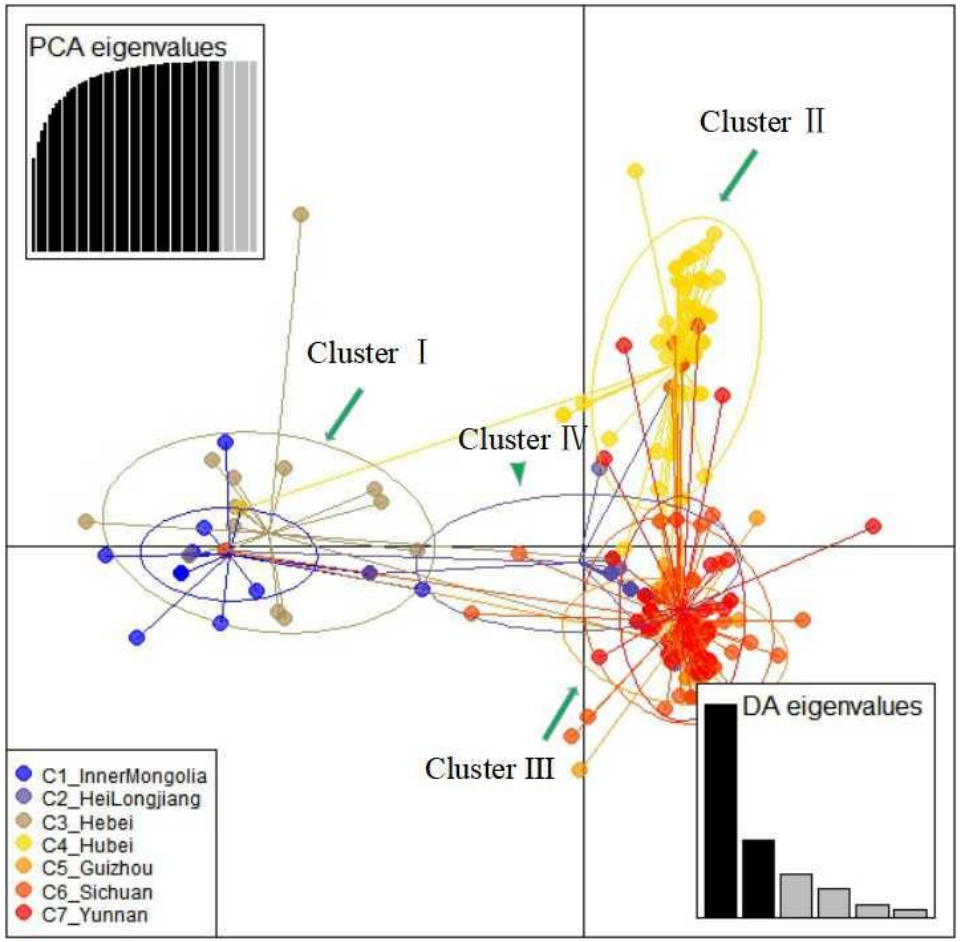
Both 13_A2 and mis_RU were found in Heilongjiang province



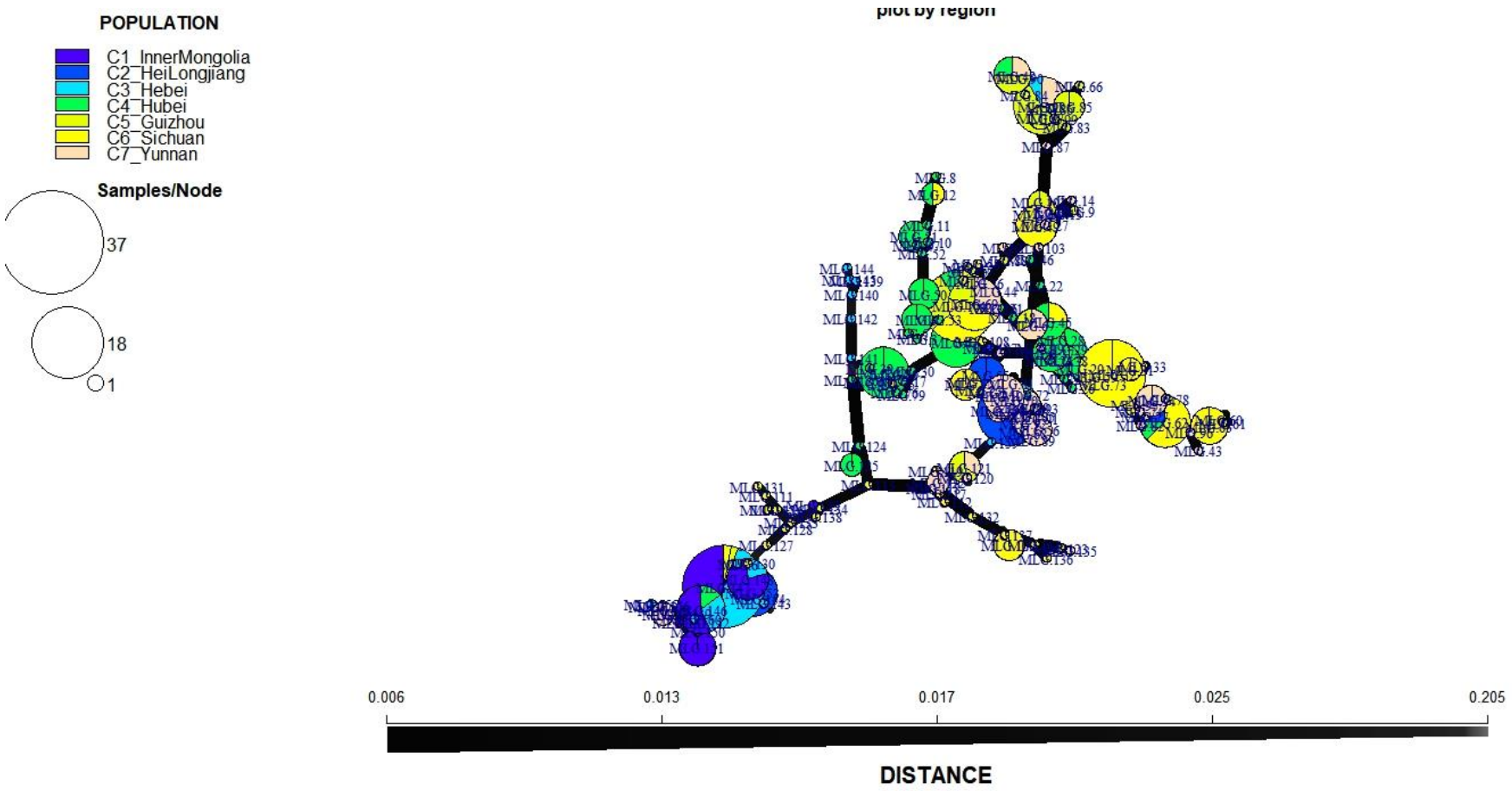
Higher MLG genotype diversity in southern regions compared with northern regions



Geographical genotype diversity was found between provinces



Geographical genotype diversity cont.



Conclusions

One dominant mating type of *P. infestans* in each province indicates that in 7 provinces in China, **sexual reproduction of *P. infestans* is limited**.

Shorter latency period and faster lesion growth rate in southern provinces indicate more **aggressive isolates in southern regions** compared with northern regions.

Major genotype 13_A2, which is widely recognized as being aggressive, is found to be associated with A2 mating type in southern regions. This result is consistent with the result of aggressiveness test.

High genotype diversity in southern regions and unique genetic compositions in different regions of China indicate a **differential *P. infestans* population structure between southern and northern provinces**.

Ongoing experiments

Conducting SSR analysis of samples from 2017

Samples were collected in 2018 and 2019, and stored to be processed

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